A Period 2 Genetic Variant Interacts with Plasma SFA to Modify Plasma Lipid Concentrations in Adults with Metabolic Syndrome1–3

Abstract

Genetic variants of Period 2 (PER2), a circadian clock gene, have been linked to metabolic syndrome (MetS). However, it is still unknown whether these genetic variants interact with the various types of plasma fatty acids. This study investigated whether common single nucleotide polymorphisms (SNPs) in the PER2 locus (rs834945 and rs2304672) interact with various classes of plasma fatty acids to modulate plasma lipid metabolism in 381 participants with MetS in the European LIPGENE study. Interestingly, the rs2304672 SNP interacted with plasma total SFA concentrations to affect fasting plasma TG, TG-rich lipoprotein (TRL-TG), total cholesterol, apoC-II, apoB, and apoB-48 concentrations (interaction <0.001–0.046). Carriers of the minor allele (GC+GG) with the highest SFA concentration (>median) had a higher plasma TG concentration (P=0.001) and higher TRL-TG (P<0.001) than the CC genotype. In addition, participants carrying the minor G allele for the rs2304672 SNP and with a higher SFA concentration (>median) had higher plasma concentrations of apo C-II (P<0.001), apo C-III (P=0.009), and apoB-48 (P=0.028) compared with the homozygotes for the major allele (CC). In summary, the rs2304672 polymorphism in the PER2 gene locus may influence lipid metabolism by interacting with the plasma total SFA concentration in participants with MetS. The understanding of these gene-nutrient interactions could help to provide a better knowledge of the pathogenesis in MetS. J. Nutr. 142: 1213–1218, 2012.

Introduction

Circadian clock systems generate and regulate circadian rhythms in concert with environmental changes to synchronize behavioral and physiological functions (1,2). Accumulating evidence indicates that disruption of different clock genes contributes to metabolic diseases such as obesity, cardiovascular diseases, metabolic syndrome (MetS)16, and cancer (3–5).
The disruption of **PER2**, one of the transcription factors from the repressor limb of the molecular clock, has been previously related to the promotion of cancer (6). In experimental studies, it was reported that disruption of the core clockwork by mutations in **PER2** increases the susceptibility of mice to spontaneous and irradiation-induced tumors (7). **PER2** disruption has also been related to different MetS components, such as abdominal obesity (8,9) and glucose metabolism, via influence on the glucose-6-phosphatase gene (10). Indeed, **PER2**−/− mice have a defective feeding rhythm, are hyperphagic and develop obesity when fed a high-fat diet (11), and have altered blood glucose homeostasis (12), demonstrating the striking influence of diet on this gene. In this context, a common strategy to examine **PER2** function in humans has relied on assessing the clinical phenotypes associated with variants in this gene. Indeed, one of the most studied **PER2** variants is **rs2304672**, which has been associated with abdominal obesity and related to attrition in weight-loss treatment, given that this variant may modulate eating behavior-related phenotypes (13,14). In addition, other **PER2** gene variations have also been studied and linked with the risk factors of MetS (15,16).

The circadian system is influenced by various stimuli, with food intake being a dominant external synchronizer (17). Particularly evident is the relationship between the circadian system and fat intake (18). Moreover, the circadian system influences fat digestion and metabolism via regulation of expression and/or activity of several metabolic enzymes and transport systems involved in the metabolism of fatty acids, which also show circadian rhythmicity (19,20). Earlier work demonstrated that there is a coordination between the **PER2** gene and specific nuclear receptors involved in lipid metabolism, such as PPARα (10). In this regard, it is known that plasma fatty acid composition may influence the development of MetS (20) and it has also been demonstrated that gene-nutrient interactions may modulate the effect of certain polymorphisms in patients with MetS (21–25). Therefore, knowledge of the influence of the interactions between genetic variants in the **PER2** gene and plasma fatty acid composition is a focus of interest. In the present study, we investigated whether genetic variations at the **PER2** locus interact with the plasma fatty acid composition to modulate lipid metabolism in patients with MetS.

### Participants and Methods

Many of the applied methods in the present article were recently published in articles about the LIPGENE cohort (22–27). Briefly, participants aged 35–70 y and with a BMI of 20–40 kg/m² were recruited for the LIPGENE dietary intervention study from 8 European countries (Ireland, UK, Norway, France, The Netherlands, Spain, Poland, and Sweden). The National Cholesterol Education Program criteria for MetS were used for the participants selection (28).

#### Biochemical measurements

The information about biochemical measurements has been previously described in the LIPGENE cohort (26). Plasma, serum, and buffy coat were prepared from 12-h fasting blood samples. The IL Test kit (Instrumentation Laboratories) was used for direct quantification of lipid concentrations (26).

#### Single nucleotide polymorphism selection and genotyping

The **rs934945** and **rs2304672** polymorphisms were genotyped at the **PER2** gene. DNA was extracted from buffy coat samples using the AutoPure LS automated system (Gentra Systems) and low-yielding samples (<10 ng) were subjected to whole-genome amplification using the REPLI-g kit (Qiagen). Genotyping was performed by Provenika. Adherence to the Hardy-Weinberg equilibrium at each single nucleotide polymorphism (SNP) locus was determined using the χ² test with 1 d.f.

### Statistical analyses

Statistical analysis was carried out using SPSS version 18.0 for Windows. Data were presented as means ± SEM for continuous variables and as frequencies or percentages for categorical variables. Differences in mean values were assessed using ANOVA and unpaired t tests. Furthermore, comparisons of frequencies between qualitative variables were carried out using the χ² test. Potential confounding factors were age, gender, BMI, and LIPGENE center of origin. Gene-nutrient interactions were examined using an SNP-fatty acid interaction term in a univariate general linear model. The effect of each SNP interacting with plasma fatty acids [total SFA, total MUFA, and total (n-6) and (n-3) PUFA] on each biochemical variable was investigated using the median of plasma fatty acids to dichotomize the sample and using the resulting groups (> or ≤ median) as a fixed factor in combination with the SNP genotypes in an ANOVA. All analyses were adjusted for potential confounders and *P < 0.05* was considered significant.

### Results

Genotype distributions did not deviate from Hardy-Weinberg expectations for **PER2** SNPs. Given the low genotype frequencies of individuals homozygous for the minor alleles, and because the analysis did not suggest a recessive mode of action, we analyzed all SNPs using 2 genotype categories (homozygotes for the major allele vs. heterozygotes combined with homozygotes for the minor allele). The 2 SNPs (**rs934945** and **rs2304672**) were included in the analysis, although the results are presented only for the **rs2304672** SNP for which significant differences were obtained. Baseline characteristics of participants and genotype frequencies are shown in Table 1. To ensure similar sample sizes for all analyses, only participants with all lipid and genetic data available were included.

We examined the effects of the **PER2** SNPs on the plasma lipid concentrations in relation to concentrations of specific types of fatty acids. The plasma fatty acid pattern (MUFA, SFA, **TABLE 1** Age, sex, BMI, and plasma biochemistry of adults with MetS according to **PER2** rs2304672 genotype (LIPGENE study)¹

<table>
<thead>
<tr>
<th></th>
<th><strong>CC</strong> (n = 328)</th>
<th><strong>GC+GG</strong> (n = 53)</th>
<th><strong>P</strong> value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>53 ± 0.50</td>
<td>56 ± 1.15</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Sex f/M, n/n</strong></td>
<td>173/155</td>
<td>32/21</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>32 ± 0.23</td>
<td>32 ± 0.61</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Plasma analytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TG, mmol/L</strong></td>
<td>1.75 ± 0.04</td>
<td>1.99 ± 0.16</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>TC, mmol/L</strong></td>
<td>5.31 ± 0.05</td>
<td>5.27 ± 0.12</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mmol/L</strong></td>
<td>1.10 ± 0.01</td>
<td>1.07 ± 0.03</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mmol/L</strong></td>
<td>3.24 ± 0.05</td>
<td>3.07 ± 0.11</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>TRL-TG, mmol/L</strong></td>
<td>0.83 ± 0.02</td>
<td>1.05 ± 0.10</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>apoA-I, g/L</strong></td>
<td>1.40 ± 0.01</td>
<td>1.38 ± 0.03</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>apoB, g/L</strong></td>
<td>1.01 ± 0.01</td>
<td>1.01 ± 0.03</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>apoB-48, mg/L</strong></td>
<td>0.76 ± 0.05</td>
<td>0.94 ± 0.18</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>apoC-II, mg/L</strong></td>
<td>45 ± 0.93</td>
<td>48 ± 3.27</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>apoC-III, mg/L</strong></td>
<td>156 ± 2.72</td>
<td>172 ± 8.71</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>PUFA, % of total lipids</strong></td>
<td>39 ± 0.38</td>
<td>38 ± 0.99</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>SFA, % of total lipids</strong></td>
<td>28 ± 0.26</td>
<td>28 ± 0.63</td>
<td>0.51</td>
</tr>
</tbody>
</table>

¹ Values are mean ± SEM. MetS, metabolic syndrome; TC, total cholesterol; TRL-TG, TG-rich lipoprotein.
and PUFA) was dichotomized according to median plasma fatty acid (≥ or ≤ median). Interestingly, the rs2304672 SNP interacted with plasma total SFA concentrations to affect fasting plasma TG, TG-rich lipoprotein (TRL-TG), total cholesterol (TC), apoC-II, apoB, and apoB-48 concentrations (P-interaction < 0.001–0.046) (Table 2). Thus, among participants with a high SFA concentration (≥ median), the minor allele for rs2304672 (GC and GG) was associated with a higher plasma TG concentration (P = 0.001) (Fig. 1A,B) and higher TRL-TG (P < 0.001) compared with homozygotes for the major allele (CC) (Fig. 2A,B). In addition, participants carrying the minor allele G for rs2304672 SNP (GC and GG) and with a high concentration of plasma SFA (≥ median) had higher apoC-II (P < 0.001), apoC-III (P = 0.009), and apoB-48 (P = 0.028) concentrations compared with the homozygotes for the major allele (CC) (Table 2). In contrast, carriers of the minor allele (GC and GG) with plasma SFA concentrations less than the median had lower plasma concentrations of fasting TG, TRL-TG, TC, apoC-II, apoC-III, apoB, and apoB-48 compared with CC participants (Table 2). There were no interactions between other plasma fatty acid types (MUFA and PUFA) and SNPs affecting plasma lipid concentrations.

### Discussion

Our study provides novel evidence indicating that genetic variations in the PER2 gene locus modulate plasma lipid concentrations in MetS patients, and this association may be influenced by the plasma fatty acid composition.

The role of the PER2 clock gene in energy metabolism and its role in the generation of metabolic rhythms has been demonstrated in mice. Per2−/− mice fed a high-fat diet developed a series of metabolic alterations related to obesity (29). In the current study, we observed that the influence of PER2 gene variants on plasma lipid concentrations may depend on circulating fatty acid proportions. Thus, among participants with higher SFA concentrations (≥ median), the minor allele (GC and GG) for the rs2304672 SNP was associated with higher fasting concentrations of TG, TRL-TG, TC, apoC-II, apoC-III, apoB, and apoB-48 compared with the homozygotes for the major allele (CC). In contrast, in this particular SNP there were no differences between genotypes when the SFA concentrations were below the median plasma concentration.

The rs2304672 SNP was previously associated with abdominal obesity and eating behavior-related phenotypes. In this report, Garault et al. (14) showed that carriers of the minor G allele for this SNP had the lowest waist-to-hips ratios compared with CC participants. However, those participants had a greater probability of attrition from a weight loss treatment program than reported by CC participants. In this regard, Yang et al. (29) showed that Per2−/− mice were hyperphagic and became obese when they consumed a high-fat diet. In addition, Lamia et al. (12) demonstrated that Per2−/− mice have altered blood glucose homeostasis. All this evidence suggests that PER2 disruption is linked to different MetS components such as abdominal obesity (8) and altered glucose metabolism (10).

It has been demonstrated that PER2 and nuclear receptors affect rhythmic transcription of output genes like glucose-6-phosphatase involved in glucose homeostasis (10). However, the mechanism underlying the associations between genetic variants of PER2 and lipid metabolism is unknown. In this regard, it has been demonstrated that PER2 coordinates circadian output to metabolic pathways by interaction with nuclear receptors, including PPARα (10). On the other hand, PPARα modulates the metabolism of TRL-TG at the stage of production and clearance of VLDL (30,31). Through the regulation of genes involved in transport and catabolism of fatty acids, PPARα regulates the availability of hepatic TG for VLDL assembly. PPARα knockout mice that consumed a high-fat diet developed higher serum total TG concentrations due to enhanced VLDL secretion compared with PPARα+/− mice that were used as controls (32). In humans, treatment with fibrates reduces TG concentration via decreased production and increased clearance of VLDL due to the induction of PPARα (33). Therefore, from our results, we hypothesize that the mechanism by which this particular SNP interacts with plasma SFA concentrations and thereby modify plasma lipid concentrations could be via PPARα. Indeed, it has been demonstrated in mice that PER2 interacts with PPARα and other nuclear receptors such as REV-ERBα and acts as a coregulator of nuclear receptor-mediated transcription (10). Thus, in response to environmental signals, PER2 could modulate biological networks related to the control of energy, glucose, and lipid metabolism given its interaction with nuclear receptors involved in these pathways (2,10,34–36).

In our current study, the main interaction between rs2304672 and plasma concentration of total SFA was with TG and TRL-

### Table 2

<table>
<thead>
<tr>
<th>Plasma analyte</th>
<th>CC (n = 159) ≤30.9 mmol/L</th>
<th>GC+GG (n = 27) &gt;30.9 mmol/L</th>
<th>High plasma SFA</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG, mmol/L</td>
<td>1.51 ± 0.06†</td>
<td>1.43 ± 0.15†</td>
<td>1.98 ± 0.06</td>
<td>0.98 ± 0.17*</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.25 ± 0.07†</td>
<td>4.85 ± 0.18†</td>
<td>5.40 ± 0.07</td>
<td>5.58 ± 0.19*</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.12 ± 0.02</td>
<td>1.12 ± 0.04</td>
<td>1.08 ± 0.02</td>
<td>0.99 ± 0.05*</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.22 ± 0.08</td>
<td>2.82 ± 0.20</td>
<td>3.26 ± 0.08</td>
<td>3.27 ± 0.21*</td>
</tr>
<tr>
<td>TRL-TG, mmol/L</td>
<td>0.68 ± 0.04†</td>
<td>0.74 ± 0.10†</td>
<td>0.96 ± 0.04</td>
<td>1.40 ± 0.10*</td>
</tr>
<tr>
<td>apoA-I, g/L</td>
<td>1.41 ± 0.01</td>
<td>1.34 ± 0.04</td>
<td>1.40 ± 0.01</td>
<td>1.35 ± 0.04*</td>
</tr>
<tr>
<td>apoB, g/L</td>
<td>1.00 ± 0.01*</td>
<td>0.91 ± 0.04†</td>
<td>1.03 ± 0.01</td>
<td>1.09 ± 0.04*</td>
</tr>
<tr>
<td>apoB-48, mg/L</td>
<td>0.76 ± 0.07</td>
<td>0.68 ± 0.18†</td>
<td>0.78 ± 0.08</td>
<td>1.37 ± 0.25*</td>
</tr>
<tr>
<td>apoC-II, mg/L</td>
<td>44 ± 1.41</td>
<td>37 ± 3.41†</td>
<td>46 ± 1.40</td>
<td>59 ± 3.58*</td>
</tr>
<tr>
<td>apoC-III, mg/L</td>
<td>144 ± 3.93†</td>
<td>145 ± 9.50†</td>
<td>167 ± 3.90</td>
<td>195 ± 9.98*</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM. †Different from corresponding high SFA, P < 0.05; *different from CC in the same SFA group, P < 0.05. TC, total cholesterol; TRL-TG, TG-rich lipoprotein.
Among participants with a high SFA concentration, the minor allele for rs2304672 (GC and GG) was associated with higher fasting plasma TG and TRL-TG concentrations compared with homozygotes for the major allele (CC). Thus, the minor G-allele genotype group may be classified as high responders to plasma concentrations of SFA and the major C allele homozygote group as low responders. These findings are reinforced by the results shown in the current study for apoB and apoC. Participants carrying the minor allele G for rs2304672 SNP (GC and GG) and with a high concentration of SFA had higher apoB-48 concentrations compared with the homozygotes for the major allele (CC), consistent with previous findings for TG and TRL-TG. Similarly, we observed in the current study that in the presence of high SFA concentrations, participants carrying the minor allele had higher apo C-II and apo C-III concentrations. In this context, it is known that apoC-II and apo C-III are important cofactors for lipoprotein-lipase (LPL) activity (activates and inhibits, respectively), which is responsible for hydrolyzing TG. Moreover, apo C-II and apo C-III are implicated in the catabolism of TRL-TG and VLDL, participate in the regulation of LPL, and tend to be increased in the postprandial state and in hypertriglyceridemia. Therefore, as previously stated, the potential mechanistic explanation for these PER2 SNP-fatty acid interactions could be mediated by the activity of LPL under the influence of PPARα. In this regard, it has been demonstrated that the PPARα gene regulates the transcription of genes involved in lipid metabolism (30) and the activation of PPARα can lead to increased LPL expression, which may lead to plasma clearance of TG and an increase in plasma HDL cholesterol concentrations (37,38). Thus, in response to environmental signals, PER2 could modulate lipid

**FIGURE 1** Effect of the interaction between the rs2304672 SNP and plasma SFA concentration (≤ or > median) on plasma TG concentrations in adults with MetS (LIPGENE study) (A). Values are mean ± SEM. *Different from CC in the same plasma SFA group, \( P < 0.05 \). Predicted plasma TG concentrations for the PER2 rs2304672 SNP depending on the plasma SFA concentration (as a continuous variable) (B). Carriers of the minor G-allele genotype were classified as high responders to plasma SFA concentration and the major C allele homozygotes as low responders. \( n = 381 \) genotyped participants. MetS, metabolic syndrome; SNP, single nucleotide polymorphism.

**FIGURE 2** Effect of the interaction between the rs2304672 SNP and plasma SFA concentration (≤ or > median) on plasma TRL-TG concentrations in adults with MetS (LIPGENE study) (A). Values are mean ± SEM. *Different from CC in the same plasma SFA group, \( P < 0.05 \). Predicted plasma TRL-TG concentrations for the PER2 rs2304672 SNP depending on the plasma SFA concentration (as a continuous variable) (B). Carriers of the minor G-allele genotype were classified as high responders to plasma SFA concentration and the major C allele homozygotes as low responders. \( n = 381 \) genotyped participants. SNP, single nucleotide polymorphism; TRL-TG, TG-rich lipoprotein.
metabolism given its interaction with nuclear receptors implicated in these pathways such as PPARα or REV-ERBs (39,40). In addition, as an alternative underlying mechanism, this specific SNP has been suggested to provoke changes in the structure of the resulting transcript that could alter the functionality of this protein (14,41).

These interactions provide strong evidence in support of the important role of PER2 as regulator of plasma lipid metabolism in humans and reinforce the importance of this gene in metabolic rhythms. Although the etiology of MetS is not fully understood, genetic factors and gene-diet interactions play an important role in its pathogenesis. In this context, the rs2304672 variant at the PER2 gene should be considered. Obviously, we need to be cautious before extrapolating our conclusions to other population and replication of our findings is essential.

In conclusion, our results demonstrate that the rs2304672 polymorphism in the PER2 gene locus may influence lipid metabolism by interacting with the plasma fatty acid composition in participants with MetS. This knowledge, together with the already demonstrated role of PER2 in abdominal obesity and impaired carbohydrate metabolism, could pave the way for a better understanding of the complex connections between circadian disruption and MetS.

Acknowledgments

Literature Cited


